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Programmed cell death 1 polymorphism in patients with rheumatoid arthritis and its impact on disease activity

Pd-1 polymorphism and rheumatoid arthritis

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Abstract

Aim: This study aimed to assess the role of the single nucleotide polymorphism PD-1.5 C/T (7209C/T) in rheumatoid arthritis (RA) development and its effect on disease activity in the Egyptian population.

Material and Methods: A case-control study was done on 240 patients diagnosed with RA and 200 age-matched healthy individuals, which considered the control group. The PD-1.5 (7209 C/T) polymorphism was analyzed by RFLP-PCR.

Results: When comparing RA patients to controls, there was a higher incidence of 7209 TT genotype. (P =0.000, OR (95% CI) =14(4-50). The 7209 T allele was highly represented in the RA group as compared to the healthy control group (P =0.0001, OR (95% CI) =2.1(1.5-3.2). We found that the 7209 TT genotype was linked to more remission and mild state disease activity (P=0.004).

Discussion: PD-1.5 TT genotype and T allele were associated with RA in Egyptians.

Keywords

Gene Polymorphism, Pd-1.5, Rheumatoid Arthritis, Autoimmune Diseases, Disease Activity.

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Introduction

Autoimmune diseases are caused by the interaction between genetic susceptibility and environmental factors. Genetic liability to autoimmune diseases is complex and includes multiple genes that organize the functions of different immune cells [1]. Human genome polymorphisms have been analyzed, and new evidence of genetic susceptibility to autoimmune diseases has been discovered. [2] RA is a chronic autoimmune disorder manifested by elongated T-cell response, which evaded the normal regulatory immune mechanisms. CD4+ T cells have been suggested as the most disease-relevant cell type in rheumatoid arthritis, and multiple genes inside RA susceptibility loci are involved in the regulation and differentiation pathways of CD4+ T cells [3].

The pathophysiology of RA is complicated and mainly results in chronic arthritis and may cause systemic affections in many individuals [4]. As the actual cause of RA is still unclear, it is hypothesized that the dysregulation in the activation of T lymphocytes has a remarkable role in auto-reactivity by destroying immune tolerance [5].

The programmed cell death 1 (PDCD1) gene, also named PD-1, is situated on the chromosomal region 2q37 and expresses PD-1 protein, a 55-kDa, is a transmembrane protein and has one extra-cellular domain Ig V-like and other cytoplasmic domain of a 97-amino acid which contains two immunotyrosine motifs, one switch and another inhibitory [6].

It also has a role in the induction and persistence of T cell tolerance, which prevents tissue damage caused by effector T cell responses [7]. The pivotal role of PD-1 in regulating immune response is discovered by studies on gene disturbance that explain the specific genotype-phenotype for autoimmune disorders [8].

The PD-1.5 gene C/T polymorphism gene at 7209 is a functional one and can affect the transcription and expression of the PD-1 protein. Genetic studies suggested that there was a link between PD-1 variants and predisposition to autoimmune disorders such as RA [9]. Although the susceptibility to autoimmune disorders has been debatable, the current study aims to assess what part PD-1.5 (7209C/T) variants play in the susceptibility to RA in the Egyptian population.

Material and Methods

240 RA patients, 196 (81.7%) females and 44 (18.3%) males, were selected for our case-control study from the rheumatology and immunology outpatient department of Mansoura University Hospital, Mansoura University, Egypt.

The diagnosis of RA was based on the ACR diagnostic criteria [10]. Two hundred healthy individuals, 172 (86%) females and 28(14%) males, who have no history of autoimmune disorders and are negative for the ANA test, were considered the control group.

We collected clinical, epidemiological, and familial RA data from all participants using medical records and questionnaires. All clinical and demographic information, the disease activity score in 28 joints (DAS28), and laboratory results for CBC, ESR, CRP, RF, and anti-citrullinated protein antibodies (ACPA) were also recorded.

Patients with organ failure, chronic infection, autoimmune diseases, and malignancy were not included in this study.

Blood Sample Collection

One ml peripheral blood sample was collected from each subject and withdrawn in an EDTA tube for fresh DNA isolation, and extracted DNA was stored at -80º C until the later use for detection of the PD-1.5 C/T polymorphism.

DNA Extraction and genotyping of PD-1.5 C/T (rs2227981) polymorphism

Genomic DNA was extracted from whole blood samples using a DNA purification kit (Qiagen GmbH, Cat. No. 51104, and Hiden, Germany) [11]. Two sets of primers were designed for each polymorphism: forward 5-ACGGCCTGCAGGACTCAC-3 and reverse 5-AGGCAGGCACATATGTG-3. PCR program was done as follows: DNA was initially denatured for 5 min at 96˚C and then 30 cycles of denaturation for 60 sec at 95˚C, primer annealing for 60 sec at 56˚C, and primer extension for 60 sec at 72˚C. Extension was finally done for 7 min at 72˚C. The PCR product was 225bp. Digestion was done with BstUI restriction enzyme. The C allele gave 172 and 53bp bands while the T allele remained 225bp, as in Figure 1.

Statistical analysis

The data was entered, cleaned, and analyzed using SPSS version 22 (IBM SPSS Statistics V22.0). Qualitative data were shown as numbers and percentages, whereas numerical data were shown as means and standard deviations. The Hardy-Weinberg Equilibrium was employed to examine the genotype and allele frequencies in the two groups. To compare numerical data, the t-test was used, while the Chi-square test was used for categorical data. P< 0.05 was considered significant at a 95% confidence interval.

Ethical Approval

This study was approved by the Ethics Committee of Mansoura University, Faculty of Medicine (Date: 2018-05-18, No: R.18.05.188).

Results

Table 1 displays clinical and biochemical information for both RA patients and the control group. The DAS28 score, ESR, CRP, RF, and ACPA values of RA patients were highly significant, but the control group's mean hemoglobin level was significantly higher. Regarding age, gender, and the distribution of body mass index (BMI), there was little variation between the control and patient groups.

Table 1. Clinical and laboratory data of RA patients and control

Items	Control (N= 200)	RA (N=240)	P-value
Age (Y)	34.29± 7.15	35.86±7.97	NS
Gender(F/M)	172/28	196/44	NS*
Disease duration(month)	--------	37.27±15.72	-----
BMI (kg/m2)	29.45±4.05	30.65±2.82	NS
CRP (mg/dl)	1.79±1.07	20.65±24.44	0.000
ESR (mm/hour)	10.11±5.87	44.72±24.21	0.000
RF(IU/ml)	18.87 ±21.72	63.57 ±66.32	0.000
ACPA(U/ml)	3.74±3.44	27.37±33.36	0.000
DAS-28	4.07±1.20	----------	-------
Hb(g/dl)	13.14±2.18	10.72±1.71	0.001

* Chi-square test, N = number, BMI = body mass index, CRP = C reactive protein, ESR = erythrocyte sedimentation rate, RF = rheumatoid factor, ACPA = anti-calcitriol protein antibodies, DAS-28 = disease activity score, and Hb = hemoglobin.

Table 2. Genotypes and alleles of PD-1.5 (7209C/T) in the RA patients and control group

Items		Controls N (%)	RA N (%)	P- value	OR (95 % CI) *
Genotypes	CC	60(30)	68(28.3)	---------	Ref (1)#
	CT	134(67)	76(31.7)	0.002	0.5(0.3-0.8)
	TT	6 (3)	96(40)	0.000	14(6-34)
Dominant Model	CT+TT	140 (70)	172 (71.7)	----	Ref (1)
	CC	60(30)	68 (28.3%)	0.7	1(0.7-1.6)
Recessive Model	CT+CC	194 (97)	144 (60)	0.002	Ref (1)
	TT	6 (3)	96(40)	0.000	21 (9-50)
Allele	C	254 (63.5)	212(44.2)	----	Ref (1)
	T	146(36.5%)	268(55.8)	0.001	2.2(1.7-2.9)

*OR 95 % CI= Odds ratio 95% confidence interval, #Ref = Reference

Table 3. Clinical and laboratory data of RA patients with various genotypes and alleles of PD-1.5

Clinical and laboratory Data	CC genotype (N=68)	CT genotype (N=76)	TT genotype (N=96)	P-value	C allele (N=212)	T allele (N=268)	P-value
Age(Y)	37.79±7.49	8.06±34.71	35.40±8.15	NS®	7.79±36.69	8.07±35.20	NS#
Gender(F/M)	27.Tem	30.Ağu	41/7	NS*	84/22	112/22	NS*
Disease duration(Y)	16.87±37.24	13.75±38.16	16.62±36.58	NS®	15.67±37.67	15.76±37.03	NS#
BMI (kg/m2)	29.64±3.94	2.21±30.07	31.31±1.85	NS®	29.76±3.11	30.38±1.41	NS#
CRP (mg/dl)	26.56±23.01	12.96±17.67	29.55±21.34	NS®	22.57±21.21	25.82±20.30	NS#
ESR (mm/ hour)	41.76 ±23.01	44.34 ±21.97	26.83±47.10	NS®	22.46±42.69	46.32±.25.39	NS#
RF(IU/ml)	77.14±67.52	59.86 ±62.75	56.91± 68.15	NS®	65.75 70.94	66.18 57.74	NS#
ACPA(U/ml)	30.63 ±3.82	28.11± 32.62	24.48 ±34.07	NS	33.10 29.72	25.51 33.45	NS#
DAS-28	1.21±4.06	4.15 ±1.31	4.00 ± 1.28	NS	1.17 4.09	1.23 4.04	NS#
Hb(g/dl)	10.07±117	10.19±1.20	10.14±2.44	NS	10.63±1.15	10.60±1.33	NS#

* Chi-square test, #student t-test, ®ANOVA test, CRP= C reactive protein, BMI= body mass index, ESR= erythrocyte sedimentation rate, RF= rheumatoid factor, ACPA = anticitrullinated protein antibodies, DAS-28 = disease activity score, Hb= hemoglobin

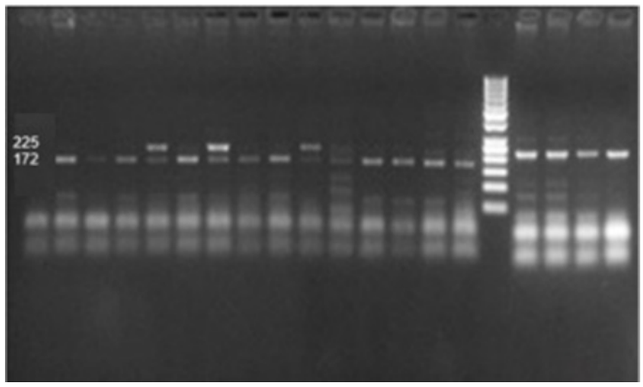

Figure 1. Enzymatic digestion of PD-1.5 (7209 C/T) gene polymorphism in different groups studied using a 50 bp size DNA ladder. T allele (225), C allele (172, 53). The 53 bp band appears faint on the gel

PCR determination of PD-1.5(7209C/T) polymorphism in RA patients showed that there was a higher frequency of 7209 TT genotype in the patient's group when compared with the controls (P =0.000, OR (95% CI) =14(4-50). In the recessive model, the risk for the development of RA was increased in individuals with TT genotype (P =0.000, OR (95% CI) =21 (6.5-71). The 7209 T allele was highly frequent in the RA group as compared to the healthy group (P=0.0001, OR (95% CI) =2.1(1.5-3.2) as illustrated in Table 2.

Various genotypes and alleles of PD-1.5 polymorphism are displayed in Table 3. There was no correlation between PD-1.5 genotypes and/or alleles and all clinical and laboratory markers in RA patients.

Discussion

The average life expectancy is reduced by three to ten years due to RA, a chronic systemic inflammatory disease that mostly affects the small joints of the hands and feet. With a 60% estimated heritability, RA is a multigene illness with a significant genetic component [3]. Genes involved in T-cell responses might be involved in the development of RA [12]. An inhibitory receptor called PD-1 controls the immune system and inhibits the onset of autoimmune diseases [13]. An increased incidence of autoimmune illnesses, including rheumatoid arthritis (RA), is strongly linked to polymorphism of the PDCD1 gene for the PD-1 protein. This finding raises the possibility that PD-1 plays a role in the pathophysiology of these diseases [8]. Many researchers looked at how PD-1 gene polymorphisms affect RA vulnerability however, the results are still unclear.

When comparing the patients' group to the controls, our research revealed a higher occurrence of the T allele and the TT genotype (P =0.000 & P =0.0001, respectively). Our results matched Susanne S et al. [14] found that the occurrence of RA in the T allele was linked to an increased vulnerability to RA, and the T allele was proven to be a significant marker of RA. Conversely, Zou et al. [9] in China did not discover any link between PD-1.5 C/T polymorphism and RA among the Chinese population.

In another research, Yuming Z et al. [9] reported that there is no significant association between PD-1.5 C/T polymorphism and the risk of RA. Moreover, the T allele of the PD1.5 (rs2227981)

C/T polymorphism was related to the RA risk among Chinese RA patients from Taiwan but not from Hong Kong. [15] An Iranian study examined the role of PD1.1 at position -538 in the promoter region of the PD-1 gene and found that this polymorphism is linked to a higher incidence of RA when compared to controls (2.9% vs. 0.7%, OR= 3.735, p=0.046) [16]. The PD-1.5 gene is situated at exon five and is considered an equivalent variation that fails to change the amino acid sequence of the PD-1 protein. This important relationship may be attributed to this variant with other PD-1 gene polymorphisms through linkage disequilibrium; this link may affect PD-1 expression at mRNA and protein levels [17]. Previous research found increased PD-1 expression in T lymphocytes [18] and increased PD-1 protein levels in RA [11]. This leads us to investigate whether PD-1 polymorphism may be responsible for altered PD-1 expression in RA. The blood levels of apoptotic markers were not investigated, which may have been considered a limitation of this study, as the serum levels of apoptotic markers may give a clearer idea about the role of PD-1 in the disease susceptibility and progression.

Belkhir et al. [19] reported that the PD1.3 (rs11568821) in Swedish patients polymorphism showed a tendency toward association with RA; however, it was not observed in a population from southeast China or populations from southern Brazil or Japan. PD 1.5 C/T polymorphism was examined in other diseases as a previous study found an association of PD 1.5 C/T polymorphism with gastric cancer in the Iranian population [20] and other autoimmune diseases like Systemic Lupus Erythematosus (SLE) in Malaysian and Indian populations [21].

The differences in the frequency of alleles and genotypes between our results and other research might be explained by the heterogeneity of RA disease, different races, different sample sizes, and the methodology by which this polymorphism was examined.

Regarding the association between PD1.5 polymorphisms and the clinical and laboratory data in the RA group, we demonstrated no correlation between PD-1 genotypes and/or alleles and all clinical and laboratory markers in RA patients. Also, we found that the TT genotype was linked to more remission and mild state disease activity (P=0.004). So, we can postulate the TT genotype is associated with a risk for RA but with milder disease.

Conclusion

We concluded that the TT genotype and T allele of PD-1.5 polymorphism may increase RA susceptibility among the Egyptian population. Other polymorphisms in the programmed cell death genes should be investigated on a large scale. Study the level of PD-1 altogether with its gene polymorphism may give better explanation of the effect of PD-1 in RA.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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